Differences in the Apparent Permeability of Spore Walls and Prothallial Cell Walls in Onoclea sensibilis

JOHN H. MILLER

The spores of *Onoclea sensibilis* L. undergo marked changes in their apparent permeability during germination. For example, spores do not stain with an acetocarmine-chloral hydrate mixture until germination has proceeded for about 20 hr (Towill & Ikuma, 1975; Fisher & Miller, 1978), whereas after that time stain is absorbed readily. During the early stages of germination, the spores of *Onoclea*, *Matteuccia struthiopteris* (L.) Tod., and other species are difficult to prepare for electron microscopy because embedding resins fail to penetrate adequately (Marengo, 1973; Gantt & Arnott, 1976; Raghavan, 1976). Spores in later stages can be processed with no difficulty. This impermeability seems to be associated with the inner spore coat (intine). Fisher and Miller (1978) noted that when the intine of *Onoclea* spores was artificially ruptured during the early stages of germination and the protoplast was directly exposed to acetocarmine-chloral hydrate, the protoplast stained rapidly, whereas no intact spores could be stained. The time at which *Matteuccia* and *Onoclea* spores normally become penetrable by embedding resins coincides with the time the intine ruptures naturally during germination (Gantt & Arnott, 1965; Marengo, 1973). If the intine of *Onoclea* is caused to open by treatment with sodium hypochlorite, even dormant spores and those in early stages of germination may be infiltrated readily with embedding resin (Bassel, Kuehnert & Miller, 1981).

Dormant spores of both *Onoclea* and *Matteuccia* have a loose outer spore coat and a thick intine, along which there is a longitudinal seam, the raphe (laesura), on the flattened, proximal face of the spore; the spore protoplast is naked within the intine (Gantt & Arnott, 1965; Bassel, Kuehnert & Miller, 1981). Germinating spores synthesize a new wall around the protoplast inside the intine between 8 and 16 hr. At the time the intine ruptures and is cast off, this new wall becomes the bounding wall of the young protonema. Clearly there is a difference in which materials will penetrate the spore intine and which will cross the normal prothallial cell wall. Carpita et al. (1979) published a method for obtaining quantitative information about the apparent capillary pore size of plant cell walls, which limits the passage of solutes. The cells are placed in a solution of a non-ionic solute having a water potential lower than that of the cells, which causes water to leave the cells. If the solute can pass through the wall, and thus the solution can be in contact with the plasma membrane, one observes plasmolysis (retraction of the protoplast from the cell wall). If, however, the solute particles are too large to penetrate the wall, exit of water from the cell causes cytorrhysis (collapse of the cell wall around the protoplast as it shrinks). I applied this technique to spores and young gametophytes of *Onoclea* and observed differences in the apparent capillary pore sizes of the spore intine, prothallial cell walls, and rhizoid walls.

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*Department of Biology, Syracuse University, Syracuse, NY 13210.

MATERIALS AND METHODS

Sporophylls of *Onoclea sensibilis* were collected in the vicinity of Syracuse, NY in the fall of 1978. They were stored in plastic bags and refrigerated until needed. The methods by which spores were isolated from the fertile fronds and general cultural methods are described in Miller and Greany (1974). An additional step was taken in which the outer spore coat was removed from the dry spores by brief treatment with sodium hypochlorite, following which the spores were redried and stored (Vogelmann & Miller, unpubl.). Removal of the brown outer spore coat made it easier to observe plasmolysis or cytorrhysis. Dormant spores were hydrated before they were used by floating them on the surface of distilled water for three hours. Young gametophytes were grown by floating spores on the surface of Knop's solution for three days in an air-conditioned growth chamber where the temperature was 26 ± 1°C and the light intensity was about 800 ft-c of continuous cool-white fluorescent illumination. After three days, the gametophytes had 3–5 vegetative cells and one primary rhizoid.

The solutes tested included NaCl, ethylene gylcol, glycerol, glucose, sucrose, polyethylene glycol-600 (PEG-600) and PEG-1000 (all chemicals from Fisher Scientific Co.). All compounds were dissolved in distilled water. The concentrations of the solutions, which are given in *Table 1*, were selected in preliminary experiments so that responses were observed within 1–5 min. Spores or prothallia were mounted directly in a drop of solution on a microscope slide and were covered with a cover slip. Observations were made with a microscope at a magnification of approximately 300 ×. Photographs were made of representative cells.

TABLE 1. INDUCTION OF PLASMOLYSIS (P) OR CYTORRHYSIS (C) BY DIFFERENT SOLUTES.

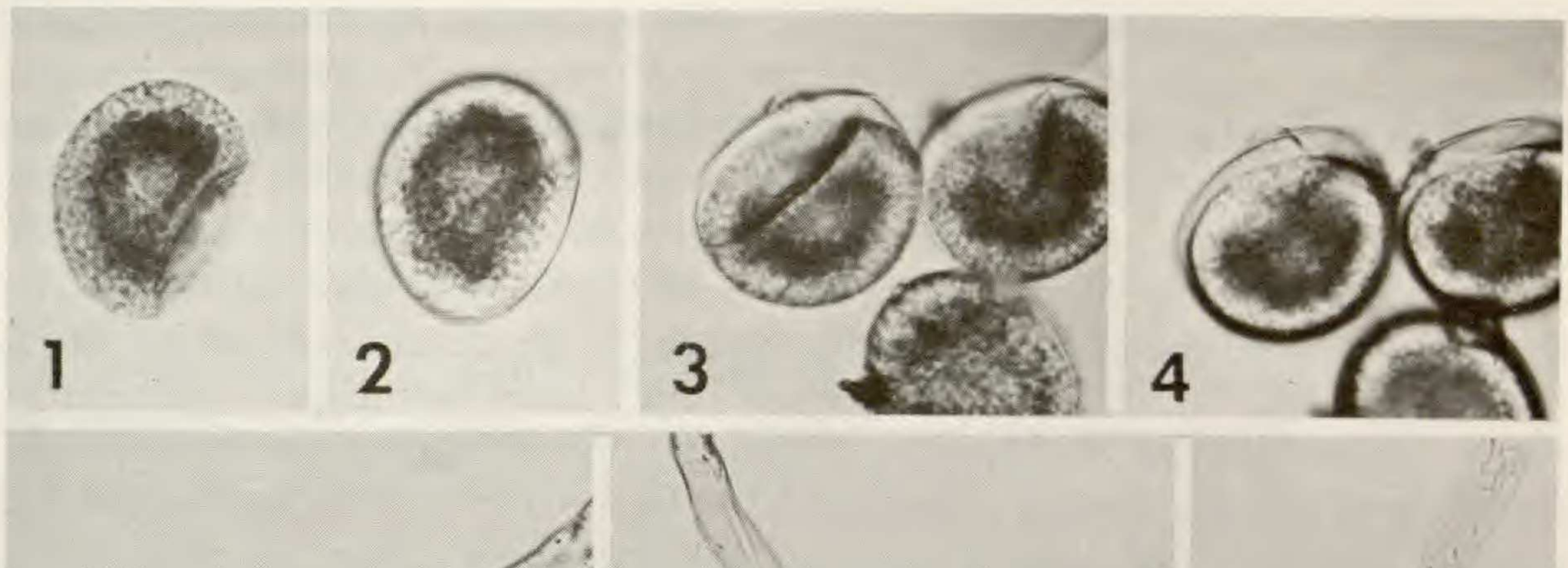
Substance and Concentration[1]	*Molecular diameter* (nm)[2]	*Spores*	*Prothallial cells*	*Rhizoids*
Ethylene glycol (50, 15)	0.45	P	P	P
Glycerol (50, 15)	0.55	P	P	P
Glucose (50, 20)	0.88	C	P	P
Sucrose (50, 20)	1.03	C	P	P
PEG-600 (50, 20)	2.9	C	P and C	P
PEG-1000 (—, 40)	3.5	—	C	C

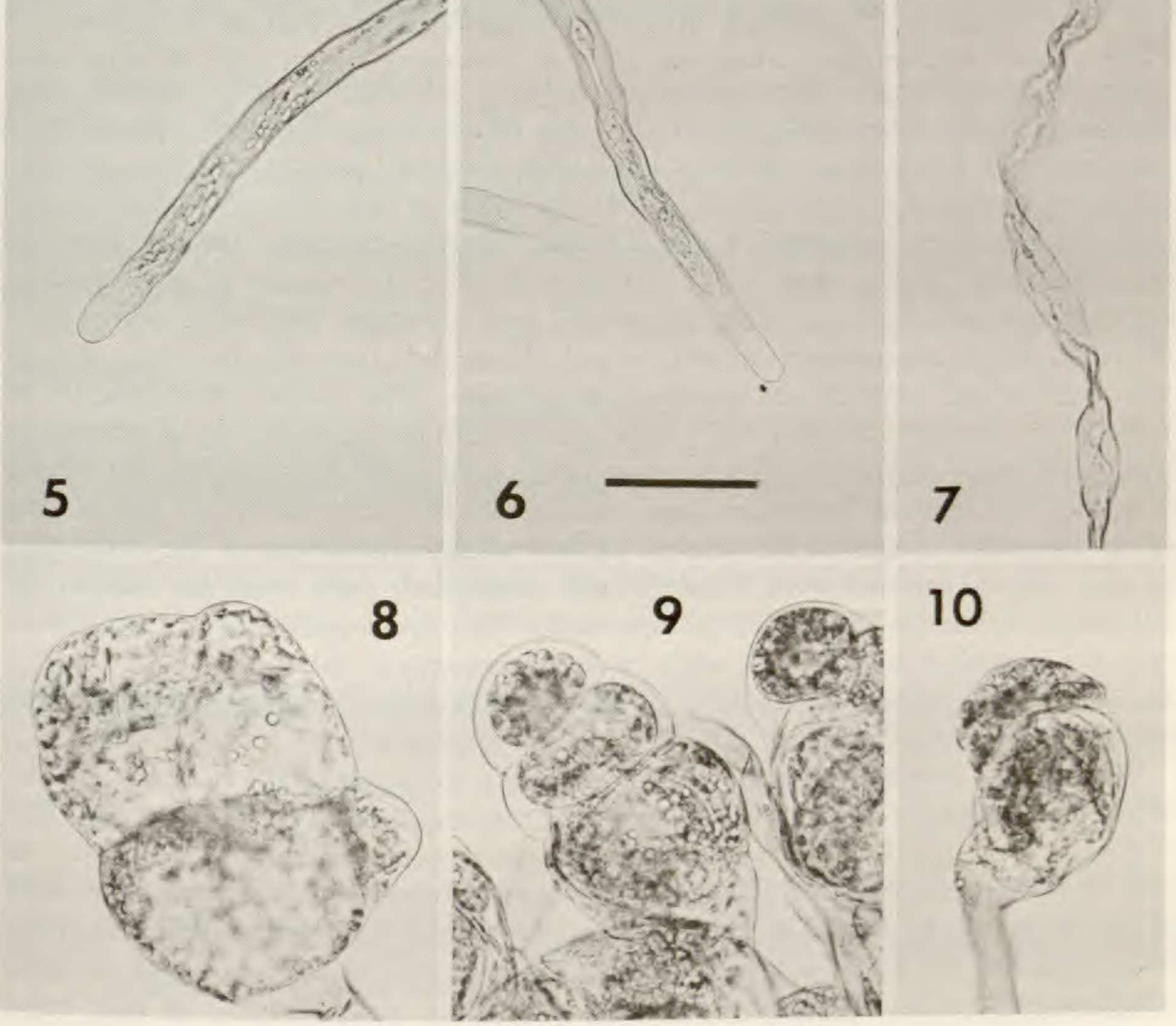
[1]% concentration (w/v). First concentration in parenthesis is for spores; second concentration is for prothallia and rhizoids.

[2]Values for ethylene glycol and glycerol from Goldstein and Solomon (1960); for glucose and sucrose from Durbin (1960); for PEG-600 and PEG-1000 from Carpita, et al. (1979).

RESULTS AND DISCUSSION

The phenomena of plasmolysis and cytorrhysis occurred in *Onoclea* spores, prothallial cells, and rhizoids *(Table 1)*. Collapse of the spore wall through cytorrhysis was demonstrated in 50% sucrose (*Figs. 1* and *2* show two views of a spore at different focal levels). Typically the spores became indented and bowl-shaped; the indentation always occurred on the proximal face (*Fig. 1*). No plasmolytic retraction of the protoplast took place at any point; even at the rim of the





Figs. 1–10. Examples of plasmolysis and cytorrhysis in spores, rhizoids, and prothallial cells of *O. sensibilis*; 50 μm scale is the same for all photographs. FIGS. 1 and 2. Cytorrhysis in a spore shown at different focal levels. FIGS. 3 and 4. Plasmolysis in spores shown at different focal levels. FIG. 5. Control rhizoid. FIG. 6. Plasmolysed rhizoid. FIG. 7. Ribbon-like rhizoid, collapsed as a result of cytorrhysis. FIG. 8. Control prothallus. FIG. 9. Plasmolysed prothallus. FIG. 10. Cytorrhysis in prothallus.

bowl the granular cytoplasm was in contact with the intine (*Fig. 2*). A 15% NaCl solution clearly induced plasmolysis (*Fig. 4*). Spore plasmolysis also was accompanied by an indentation of the proximal spore face (*Fig. 3*), since there appeared to be a firm adhesion of the protoplast to the intine in the area of the raphe, and when the protoplast became plasmolysed, the intine was drawn in at that point. A control rhizoid is shown in *Fig. 5*, and plasmolysis in 20% glucose is illustrated in *Fig. 6*. Cytorrhysis in a rhizoid in 40% PEG-1000 resulted in the collapse of the cell into a ribbon form (*Fig. 7*). The vegetative portion of a normal prothallus is pictured in *Fig. 8*. Plasmolysis gave the appearance shown in *Fig. 9*, whereas the collapse and crumpling of the cells through cytorrhysis is shown in *Fig. 10*. The figures give the pictorial definition of the terms plasmolysis and cytorrhysis as they are used in this paper.

The main results are summarized in *Table 1*. Prothallial cells showed only plasmolysis with compounds up to the size of sucrose. PEG-600 caused both cytorrhysis and plasmolysis, whereas PEG-1000 produced pure cytorrhysis. Following the reasoning of Carpita, et al. (1979), the limiting pore size of prothallial cell walls appears to be between 2.9 and 3.5 nm. This is somewhat smaller than the values found by Carpita, et al. (1979) for the cells of several species of angiosperms. Rhizoids appear to have a slightly larger wall pore size than prothallial cells, since PEG-600, which caused both cytorrhysis and plasmolysis in prothallial cells, caused only plasmolysis without cell collapse in rhizoids. The overall permeability of rhizoids was shown by Smith (1972) to be greater than the permeability of prothallial cells of *Polypodium vulgare*. His measurements were made by following the uptake of a vital dye into the protoplast, and thus reflect the permeability of the plasma membrane. The results of the present study indicate that some of the difference between the permeability of rhizoids and prothallial cells may be caused by differences in the permeabilities of their walls. The spore intine clearly was much more impermeable than the cell walls of the gametophyte. Glycerol was the largest molecule which caused plasmolysis of the spore protoplast; glucose induced pure cytorrhysis. The capillary pore size of the intine appeared to be less than 0.8 nm.

Spores were visibly affected only by higher concentrations of each of the substances than were required to produce effects in rhizoids or prothallial cells. This may reflect the fact that the spore cytoplasm is very dense and non-vacuolate, as seen in electron micrographs (Bassel, Kuehnert & Miller, 1981). More of the water of the spore may be bound in the hydration of proteins, for example, and relatively little available for free osmotic exchange. The same concentrations of osmotic solutions which plasmolysed or collapsed prothallial cells within 1–5 min acted much more rapidly on rhizoids. In each test, rhizoids were affected in less than 30 sec, the time necessary to prepare the sample and make the first observation. This rapidity is probably another reflection of the greater permeability of rhizoids. When young gametophytes were placed in plasmolysing solutions, the basal cell of the plant was affected first, followed by the intermediate and more apical cells. Deplasmolysis occurred in prothallial cells and rhizoids which were plasmolysed in certain of the solutions. Both cell types deplasmolysed completely within 15 min after immersion in ethylene glycol. Prothallial cells were deplasmolysed completely in two hr in glycerol and were partially deplasmolysed in the same time in glucose.

Rhizoids showed only a partial recovery in either of these two compounds. Plasmolysis appeared to be permanent in solutions of any of the large substances. The instances of deplasmolysis indicate that the plasma membranes of the cells were permeable to the plasmolysing solute, and enough was taken up eventually to reverse the flow and cause an influx of water into the protoplasts.

The results which are presented in this paper support the idea that the low permeability of *Onoclea* spores results from the permeability properties of the intine. The estimated capillary pore size of the intine is only about one quarter that of prothallial cells and rhizoids. The intine should play a major role in determining the entry and exit of materials into and from the spore during the first stages of germination before the intine is ruptured. Some aspects of permeability seem not to be explicable on this basis. Vogelmann (1980), for example, showed that colchicine, griseofulvin, and isopropyl N-chlorophenyl carbamate produce striking effects on spore germination, and each appears to enter the spore before the time of intine rupture, although one would expect them to be excluded on the basis of their size. One possible explanation for this type of anomaly is the suggestion by Carpita, et al. (1979) that a small number of larger pores might provide access to the protoplast by larger molecules, whereas osmotic effects may be governed by the more abundant smaller pores.

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